ZOOLOGICAL SCIENCE 3: 699–706 (1986)

Rumen Ciliate Protozoal Fauna of Zebu Cattle (*Bos taurus indicus*) in Sri Lanka, with the Description of a New Species, *Diplodinium sinhalicum* sp. nov.

Soichi Imai

Department of Parasitology, Nippon Veterinary and Zootechnical College, Musashino, Tokyo 180, Japan

ABSTRACT—Rumen ciliate composition was surveyed on the zebu cattle in Sri Lanka. As the result of survey, 16 genera including 53 species with 19 formae were detected. Of them, one new diplodiniid species was recognized, then described as *Diplodinium sinhalicum* sp. nov. From the comparison of their fauna with that of the domestic animals kept in neighbouring tropical area, every composition indicated high similarity. However, some of them were the species which have been reported not from tropical area but from northern area such as U.S.S.R. and Japan. From the results, the origin of host zebu cattle examined was discussed. The average density of ciliates per 1 ml of the rumen fluid was 2.9 $\times 10^4$, and the number of ciliate species per head of host was 18.4.

INTRODUCTION

It is known that the rumen ciliate faunae vary with the species of hosts and/or distributing area of hosts [1]. Extensive studies on the rumen microfaunae have been made in different areas of the world [2]. On the microfaunae of zebu cattle which is one of the most popular domestic ruminants around the tropical area, the surveys have been performed on the animals in Philippines [3] and Thailand [4] by us, and in India and Sri Lanka by Kofoid and MacLennan [5–7].

The present investigation was carried out to clarify the rumen ciliate fauna of the zebu cattle in Sri Lanka, which has been left out of investigation since the reports by Kofoid and MacLennan [5–7], as one of the studies to provide informations for the discussion on the phylogenetic relation among the rumen ciliates and the host ruminants. The ciliate composition obtained was taxonomically discussed with the classification by Kofoid and MacLennan [5–7] and compared with those of the animals kept in neighbouring areas. One new species found in this examination is also described.

Accepted March 26, 1986
Received February 17, 1986

MATERIALS AND METHODS

Rumen contents examined were collected from 20 "Sinhala" cattle slaughtered in the abattoir in Kandy, Sri Lanka in November 1982. The animals had been fed continuously on the wild true grasses. The contents collected were immediately fixed with twice volume of MFS (methylgreen-formalin-saline) solution [2], sealed up, and brought to the laboratory. Then, the samples were added to further with 3 times volume of MFS solution and examined under a light microscope. Identification of genera and species of the ciliates was mainly in accordance with the description published by Ogimoto and Imai [2]. The ciliates not described in their description were identified in conformity to Kofoid and MacLennan [5–7] and Dogiel [8]. The orientation of ciliates for description of the new diplodiniid species was in accordance with the previous paper [9]; that is, the side closest to the macronucleus and contractile vacuole was called the "left side" with the opposite side "right side", and the other sides were called the "upper" and "lower" side. Counting procedure for the density of total ciliates and for generic composition was according to the previous paper [9].

A part of the samples from which the new species was recognized was stained with Mayer's

hematoxylin staining and prepared as permanent slides for holotype and paratypes.

RESULTS

1. *Description of a new species*

One new ciliate was detected from a sample on the course of survey. The morphological characters of the new species are as follows;

Diplodinium sinhalicum sp. nov.
(Figs. 1 and 2)

Diagnosis: Body elliptical to rectangular, 55–72×37–44 μm; a length to width ratio 1.64; with two heavy caudal spines; macronucleus with thick boomerang-like shape at the anterior left side of body near anterior contractile vacuole.

Description: Viewed from the upper or lower side, the body is elliptical to rectangular, but posterior part of body is slightly slender. The body is compressed upward and downward. There are two heavy caudal spines which are almost the same size at the posterior end of body. Shape and size of the caudal spine indicate considerable variation. A clear and relatively large operculum is situated at the top of body. Oral and left ciliary zones in the retracted condition are situated in apparent triangular chambers pinching the operculum. The chamber of oral ciliary zone is larger than that of left ciliary zone. Esophagus is relatively wide and funnel-shaped extending leftward. Rectum is clear and forms a narrow slit extending a relatively long distance along the inner border of ectoplasm. Cytoproct opens just left to the right spine. A macronucleus lies at the left side to center. Its anterior half bends almost vertically to right side. An elliptical micronucleus is situated in a slight depression in the left surface of the macronucleus, in the region where the macronucleus is bent rightward. Two contractile vacuoles lie tandem in ectoplasm at the left side of macronucleus. Anterior contractile vacuole is situated at the same level as that of the center of macronucleus and the posterior vacuole is smaller than the anterior one.

Measurement of 20 individuals of this species is as follows. In the description of body length, the size of caudal spine is not contained.

	Mean	SD	Range
Length (μm)	54.8	3.0	49.0 –58.9
Width (μm)	41.7	1.9	36.8 –44.1
Caudal spine (μm)	11.0	3.4	3.5 –14.6
L/W	1.64	0.05	1.53– 1.76

Type host and locality: Zebu cattle, *Bos taurus indicus*, in Kandy, Sri Lanka.

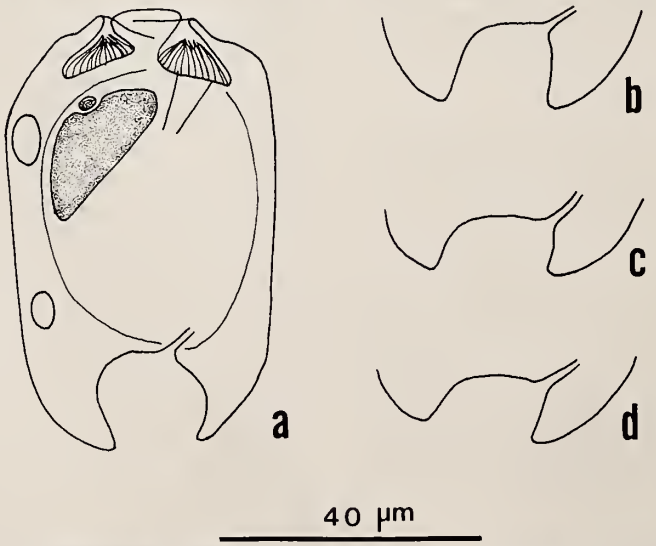

Fig. 1. *Diplodinium sinhalicum* sp. nov. a: Upper view of whole body. b–d: Variation of the caudal spines.

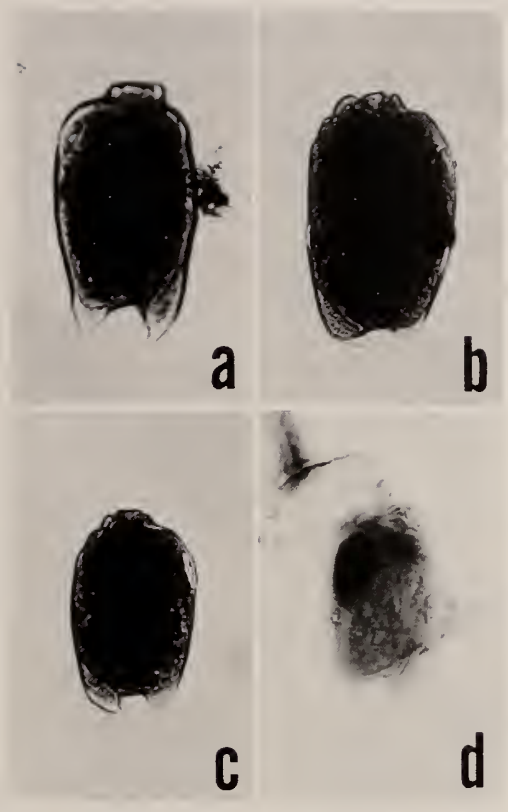

FIG. 2. Light micrographs of *Diplodinium sinhalicum* sp. nov. a–c: Upper (a) or lower (b and c) views of specimens fixed and stained with MFS solution. Note the variation of caudal spine. d: Upper view of a specimen stained with Mayer's hematoxylin. Magnification is ×450 in a and b, and ×400 in c and d.

Habitat: Rumen.

Occurrence: *D. sinhalicum* was detected in 10.0% of the zebu cattle surveyed.

Specimens deposited: Holotype and 4 paratypes on slides. They are deposited in the Department of Parasitology, Nippon Veterinary and Zootechnical College, Musashino, Tokyo, Japan.

Remarks: This species is one of the small diplodiniid species, and its shape indicates the presence of no closely related species in the genus *Diplodinium*. Most of the species belonging to the genus *Diplodinium* are over 60 μm in length, and only few are smaller. *Diplodinium polygonale* (Dogiel, 1925) is one of the smallest species in the genus, of which the size is 32–38 μm in length [8], but its morphology is easily distinguished from that of the present new species. In regard of possession of two heavy caudal spines, this new species is common with *D. anisacanthum diacanthum* Dogiel, 1927 [8], but it is also easily discriminated from *D. sinhalicum* in the points of the fairly different body shape and size. On the other hand, Kofoid and MacLennan [6] described *Eremoplastron magnodentatum* from the zebu cattle in India and Sri Lanka. The body shape of the species is similar to the present species. It is, however, clearly distinguished from the present new species by the body size and existence of one slender skeletal plate by which the genus *Eremoplastron* is characterized.

2. Composition of rumen ciliates

Genera, species, formae if presented, and the frequencies of appearance (No. of the hosts in which the species was detected/No. of examined hosts) of the ciliates found in this examination are shown in Table 1. Those were 16 genera containing 53 species with 19 formae. Of the ciliate species, *Dasytricha ruminantium, Entodinium exiguum, E. longinucleatum longinucleatum, E. biconcavum, Eudiplodinium maggii, Eremoplastron bovis* and *Ostracodinium obtusum* were the most predominant, the frequencies of appearance of which were over 70% in the animals examined. In addition, over a half of the zebu cattle had *Isotricha intestinalis, Entodinium simplex, E. nanellum, E. dubardi, E. ovinum, E. chatterjeei* and *Ostracodinium trivesiculatum*.

Table 2 shows the percentage composition of genera detected in this examination. The percentage occupied by the ciliates of *Entodinium* was the highest, the ratio of which was 63.5% on average. The ciliates of *Diplodinium* indicated next high composition, which value was 11.6%. Of other genera, *Dasytricha, Eremoplastron, Eudiplodinium* and *Ostracodinium* showed relatively high ratios.

The average number of species per head of host and the total ciliate density per milliliter of rumen fluid of the animals examined are shown in Table 3. The average number of ciliate species was 18.4, and the average total ciliate number was 2.9×10^4/ml.

Table 1. Species of rumen ciliate protozoa found from the zebu cattle and their frequency of appearance

Family	Species	Frequency of appearance (%)
Buetschliidae	*Parabundleia*	
	ruminantium Imai et Ogimoto	15.0
	Polymorphella	
	bovis Imai	5.0
Isotrichidae	*Dasytricha*	
	ruminantium Schuberg	80.0
	Isotricha	
	intestinalis Stein	65.0
	prostoma Stein	45.0
	Oligoisotricha	
	bubali (Dogiel)	10.0
Blepharocorythidae	*Charonina*	
	ventriculi (Jameson)	15.0
Ophryoscolecidae		
Entodiniinae	*Entodinium*	
	biconcavum Kofoid et MacLennan	75.0
	exiguum Dogiel	75.0
	longinucleatum Dogiel	
	f. *longinucleatum* Dogiel	75.0
	f. *spinonucleatum* Dehority	40.0
	f. *acutonucleatum* Kofoid et MacLennan	25.0
	nanellum Dogiel	65.0
	simplex Dogiel	65.0
	ovinum Dogiel	60.0
	chatterjeei Das-Gupta	55.0
	dubardi Buisson	50.0
	bovis Wertheim	45.0
	parvum Buisson	
	f. *parvum* Buisson	45.0
	f. *monospinosum* Imai et Ogimoto	35.0
	rostratum Fiorentini	40.0
	caudatum Stein	
	f. *lobosospinosum* Dogiel	40.0
	f. *caudatum* Stein	35.0
	minimum Schuberg	30.0
	ekendrae Das-Gupta	25.0
	rhomboideum Kofoid et MacLennan	25.0
	indicum Kofoid et MacLennan	20.0
	bimastus Dogiel	20.0
	anteronucleatum Dogiel	15.0
	bifidum (Dogiel)	15.0
	gibberosum Kofoid et MacLennan	15.0
	bursa Stein	10.0
	fujitai Imai	10.0
	aculeatum Kofoid et MacLennan	5.0
	javanicum Imai	5.0

(Table 1. Continued)

Family	Species	Frequency of appearance (%)
Diplodiniinae	*Diplodinium*	
	polygonale (Dogiel)	40.0
	anisacanthum da Cunha	
	f. *anacanthum* Dogiel	35.0
	f. *monacanthum* Dogiel	35.0
	f. *tetracanthum* Dogiel	25.0
	f. *anisacanthum* da Cunha	20.0
	f. *triacanthum* Dogiel	15.0
	f. *pentacanthum* Dogiel	15.0
	f. *diacanthum* Dogiel	10.0
	dentatum Stein	30.0
	psittaceum Dogiel	10.0
	sinhalicum sp. nov.	10.0
	minor (Dogiel)	5.0
	Eodinium	
	lobatum Kofoid et MacLennan	20.0
	posterovesiculatum (Dogiel)	10.0
	Eremoplastron	
	bovis (Dogiel)	70.0
	bubalus Dehority	35.0
	rostratum (Fiorentini)	20.0
	monolobum (Dogiel)	5.0
	dilobum (Dogiel)	5.0
	Eudiplodinium	
	maggii (Fiorentini)	75.0
	Diploplastron	
	affine (Dogiel)	5.0
	Polyplastron	
	multivesiculatum (Dogiel et Fedorowa)	5.0
	Metadinium	
	medium Awerinzew et Mutafowa	45.0
	Ostracodinium	
	obtusum (Dogiel et Fedorowa)	70.0
	trivesiculatum Kofoid et MacLennan	60.0
	gracile (Dogiel)	35.0
	mammosum (Railliet)	5.0
Ophryoscolecinae	*Epidinium*	
	ecaudatum (Fiorentini)	
	f. *caudatum* Fiorentini	15.0
	f. *cattanei* Fiorentini	15.0
	f. *ecaudatum* Fiorentini	10.0
	f. *eberleini* da Cunha	10.0
	f. *tetracaudatum* Sharp	5.0
Total genera, species and formae		16 genera 53 species 19 formae

Table 2. Generic composition (%) of the rumen ciliate protozoa in the zebu cattle in Sri Lanka*

Genus	Mean	Range
Polymorphella	<0.1	0– 1.5
Parabundleia	0.2	0– 1.5
Dasytricha	7.1	0–45.5
Isotricha	2.0	0–16.5
Oligoisotricha	0.3	0– 3.5
Charonina	0.4	0– 1.5
Entodinium	63.5	21.5–93.5
Diplodinium	11.6	0–39.5
Eodinium	0.9	0– 4.5
Eremoplastron	5.1	0–19.0
Eudiplodinium	3.7	0–18.0
Diploplastron	0.1	0– 2.0
Polyplastron	<0.1	0– 1.0
Metadinium	0.5	0– 2.5
Ostracodinium	3.6	0–15.0
Epidinium	1.0	0– 6.5

* n=20

Table 3. Average number of species appeared and average ciliate density in the zebu cattle in Sri Lanka*

Number of species			Ciliate density (log_{10}/ml)		
Mean	SD	Range	Mean	SD	Range
18.4	7.3	6–29	4.46	0.69	2.9–5.5

* n=20

DISCUSSION

Zebu cattle have widely been kept as domestic ruminants around the tropical area, and their rumen ciliate compositions have been reported on those in Philippines [3], Thailand [4], India and Sri Lanka [5–7], China [10] and Senegal [11]. Kofoid and MacLennan [5–7] described 45 entodiniomorphid species from the 4 zebu cattle in Sri Lanka. However, some of the species described by them were extremely indistinct in the difference to already known species, and some were described as independent species based on the caudal spine as the important character in spite of its morphological instability [2]. So that, they were adjusted in conformity to our taxonomical standard [2] as shown in Table 4. After the adjustment, the number of species detected by Kofoid and MacLennan were 38 species with 9 formae. Of them, 13 species with 3 formae were not detected in the present examination. This difference may be due to the difference of the race of zebu cattle surveyed; i.e., "Sinhala" race in the present examination and "Cingalese" and "Indian" races in Kofoid and MacLennan's survey [5]. Consequently, the number of species detected from the zebu cattle in Sri Lanka was resulted in 66 species with 22 formae in all. As compared this ciliate composition with those of the zebu cattle in other areas, which were described previously [3–7, 10, 11], every composition indicated high similarity,

Table 4. Species names considered as synonyms of the ciliates detected by Kofoid and MacLennan

Original name by Kofoid and MacLennan [5–7]	Adjusted name by us	Reference
Entodinium ellipsoideum	*Entodinium bursa*	[5]
Entodinium acutonucleatum	*Entodinium longinucleatum acutonucleatum*	[12]
Entodinium ovoideum	*Entodinium ovinum*	[5]
Eremoplastron rotundum	*Eremoplastron bovis*	[6, 8]
Eremoplastron brevispinum	*Eremoplastron dilobum*	[6, 8]
Epidinium caudatum	*Epidinium ecaudatum caudatum*	[2]
Epidinium bicaudatum	*Epidinium ecaudatum bicaudatum*	[2]
Epidinium tricaudatum	*Epidinium ecaudatum tricaudatum*	[2]
Epidinium quadricaudatum	*Epidinium ecaudatum quadricaudatum*	[2]
Epidinium cattanei	*Epidinium ecaudatum cattanei*	[2]
Epidinium eberleini	*Epidinium ecaudatum eberleini*	[2]

especially of those in neighbouring area such as India, Philippines and Thailand; i.e., their common ratios were 94.7% (36 of 38 species) from Indian cattle, 92.3% (24 of 26 species) from Philippine cattle, and 83.9% (47 of 56 species) from Thailander cattle, respectively. On the other hand, 35 of 46 species (76.1%) from Chinese cattle and 24 of 33 species (72.7%) from Senegalese cattle were common with that from the zebu cattle in Sri Lanka. In Indonesia, Bali cattle which were domesticated from wild Banteng, have been kept as domestic ruminants, and 15 genera including 45 species have been reported from them [9]. Of the species, 40 species (88.9%) were common with that in the present examination. These results might indicate the presence of geographical distribution of the rumen ciliates depending on the opportunity of chance of transfaunation as mentioned also in the previous paper [9].

Of the ciliate species detected predominatingly in this examination, most except *Entodinium biconcavum, E. chatterjeei* and *Ostracodinium trivesiculatum* are fairly common species, or they have been widely detected from various species of hosts and their habitating areas [2]. As previously mentioned by the author [9], these species might be cosmopolitan ones because of their high frequencies of appearance and high opportunity of infection to other hosts. In contrast, the 3 species, *E. biconcavum, E. chatterjeei* and *O. trivesiculatum,* have relatively narrow distribution but detected from the domestic ruminants in tropical area in relatively common [2–4, 9].

On the other hand, some species such as *Parabundleia ruminantium, Polymorphella bovis, Entodinium indicum, E. fujitai, E. javanicum, E. gibberosum* and *Diplodinium psittaceum* in addition to *D. sinhalicum* newly described, have been detected from strongly limited area. Of them, former 5 species have been found from the hosts in Southeast Asia, Thailand [4], Indonesia [9] and/or Taiwan [13]. *E. gibberosum* has been detected only from the zebu cattle in Sri Lanka [5] and the water buffalo in Brazil, which had been derived from Taiwan [14]. *D. psittaceum* has been noticed from the animals in Sri Lanka [6], China [10], Senegal [11] and U.S.S.R. [8], but not in other Southeast Asia.

In contrast, the present examination elucidated the presence of *Entodinium bursa, Diploplastron affine* and *Polyplastron multivesiculatum,* which had been reported not from the hosts in Southeast Asia but commonly from the hosts in northern area such as U.S.S.R. [8] and Japan [15].

This result suggests that the ciliate fauna of zebu cattle in Sri Lanka is strongly affected by those of the animals in tropical area because the ciliate species detected in common in those areas were apparently predominant, but influence of the faunae of the animals in northern area cannot be disregarded because of the presence of the characteristic species.

The animals examined in this survey are named as "Sinhala" cattle and thought to be carried in Sri Lanka in B.C. 6th century by the Sinhalese people who immigrated from northern India [16]. From the facts, it indicates a possibility that the ciliate fauna of the animals in Sri Lanka has been formed in the ciliates in tropical area, as well as that of Thailand and Indonesia, and those in northern Asia and Europe. The results that some ciliates, such as *Entodinium tsunodai, E. bubalum, Ostracodinium tiete* and *Enoploplastron triloricatum,* were detected both from the animals in Indonesia [9] and Thailand [4], but not from the zebu cattle in Sri Lanka, in contrast, some others, such as *Entodinium gibberosum, Diplodinium psittaceum* and *D. sinhalicum,* had the reverse relation in spite of their near location, may indicate a difference of the origin of zebu cattle in Sri Lanka from the animals in Southeast Asia. In the same point of view, the Senegalese zebu cattle [11] may be affected in the rumen ciliate fauna by the animals in northern area.

The percentage composition of genera detected in this examination resembled those in the domestic animals in Indonesia [9]. It is known that the generic composition varies with the feeding condition of host [17], and that the ratio occupied by the ciliates of the genus *Entodinium* becomes higher when the host is fed with concentrates-rich ratios [1]. For instance, the composition ratios of *Entodinium* and *Diplodinium* have been reported to be 86.5% and 1.7% on average, respectively, in the Japanese cattle fed mainly with both dry grasses and concentrates [15]. Relatively low ratio

of entodiniids and high ratio of diplodiniids in the ciliate composition of ruminants in tropical area would be one of the characteristics in the animals fed only with roughage.

Regarding the number of species per head of host, Kofoid and MacLennan [5–7] reported 24.3 species per host on average from 4 zebu cattle in Sri Lanka. In the zebu cattle in other area, the average number of species have been reported to be 26.1 in Thailand [4] and 16.5 in Philippines [3]. It has been also reported that the values in cattle and water buffalo have generally poorer ciliate composition [3, 4, 13, 15] than in the zebu cattle. The result obtained in the present examination supported this tendency. On the other hand, the average ciliate number obtained in this survey was similar to the values in the domestic ruminants kept in the neighbouring tropical area, such as Indonesia [9], Thailand [4] and Philippines [3], which were apparently lower than in the Japanese cattle [15]. Such low values in the tropical ruminants would be due to the feeding condition of hosts as mentioned in the previous paper [9].

ACKNOWLEDGMENTS

The author wishes to thank Dr. N. Taira, of the Kyushu Branch Laboratory, National Institute of Animal Health, for cooperating in the collection of the samples. Thanks are also due to Dr. M. Kinoshita, of the Department of Parasitology, Nippon Veterinary and Zootechnical College, for his technical assistance.

This work was supported in part by Grant-in-Aid No. 58560298 for Scientific Research from the Ministry of the Education, Science and Culture, Japan.

REFERENCES

1 Hungate, R. E. (1966) Rumen and Its Microbes. Academic Press, New York, 533 pp.

2 Ogimoto, K. and Imai, S. (1981) Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo, 231 pp.

3 Shimizu, M., Kinoshita, M., Fujita, J. and Imai, S. (1983) Rumen ciliate protozoal fauna and composition of the zebu cattle, *Bos indicus*, and water buffalo, *Bubalus bubalis*, in Philippines. Bull. Nippon Vet. Zootech. Coll., **32**: 83–88.

4 Imai, S. and Ogimoto, K. (1984) Rumen ciliate protozoal fauna and bacterial flora of the zebu cattle (*Bos indicus*) and the water buffalo (*Bubalus bubalis*) in Thailand. Jpn. J. Zootech. Sci., **55**: 576–583.

5 Kofoid, C. A. and MacLennan, R. F. (1930) Ciliates from *Bos indicus* Linn. 1. The genus *Entodinium* Stein. Univ. Calif. Publ. Zool., **33**: 471–544.

6 Kofoid, C. A. and MacLennan, R. F. (1932) Ciliates from *Bos indicus* Linn. 2. A revision of *Diplodinium* Schuberg. Univ. Calif. Publ. Zool., **37**: 53–152.

7 Kofoid, C. A. and MacLennan, R. F. (1933) Ciliates from *Bos indicus* Linn. 3. *Epidinium* Crawley, *Epiplastron* gen. nov., and *Ophryoscolex* Stein. Univ. Calif. Publ. Zool., **39**: 1–33.

8 Dogiel, V. A. (1927) Monographie der Familie Ophryoscolecidae. Arch. Protistenkd., **59**: 1–288.

9 Imai, S. (1985) Rumen ciliate protozoal faunae of Bali cattle (*Bos javanicus domesticus*) and water buffalo (*Bubalus bubalis*) in Indonesia, with the description of a new species, *Entodinium javanicum* sp. nov. Zool. Sci., **2**: 591–600.

10 Hsiung, T.-S. (1932) A general survey of the protozoan fauna of the rumen of the Chinese cattle. Bull. Fan Mem. Inst. Biol., **3**: 87–107.

11 Bonhomme-Florentin, A., Blancou, J. and Latteur, B. (1978) Étude des variations saisonnières de la microfaune du rumen de zebus. Protistologica, **14**: 283–289.

12 Imai, S. and Ogimoto, K. (1983) *Parabundleia ruminantium* gen. n., sp. n., *Diplodinium mahidoli* sp. n. with two formae, and *Entodinium parvum* forma *monospinosum* forma n. from the zebu cattle (*Bos indicus* L., 1758) in Thailand. Jpn. J. Vet. Sci., **45**: 585–591.

13 Imai, S., Chang, C.-H., Wang, J.-S., Ogimoto, K. and Fujita, J. (1981) Rumen ciliate protozoal fauna of the water buffalo (*Bubalus bubalis*) in Taiwan. Bull. Nippon Vet. Zootech. Coll., **29**: 77–81.

14 Dehority, B. A. (1979) Ciliate protozoa in the rumen of Brazilian water buffalo, *Bubalus bubalis* Linnaeus. J. Protozool., **26**: 536–544.

15 Imai, S., Shimizu, M., Kinoshita, M., Toguchi, M., Ishii, T. and Fujita, J. (1982) Rumen ciliate protozoal fauna and composition of the cattle in Japan. Bull. Nippon Vet. Zootech. Coll., **31**: 70–74. (In Japanese with English summary)

16 Rouse, J. E. (1970) World Cattle. Univ. Oklahoma Press, Norman, Vol. 2, pp. 867–879.

17 Warner, A. C. I. (1962) Some factors influencing the rumen microbial population. J. Gen. Microbiol., **28**: 129–146.